Cytogenetical characterization of six orb-weaver species and review of cytogenetical data for Araneidae

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Abstract. The family Araneidae is the third largest among spiders and the third most studied from a cytogenetical point of view. In spite of this, only 2% of all araneids have been karyotyped. The majority of araneids analyzed possess $2n = 24$ chromosomes in males; however, the study of additional species could reveal unusual karyotype characteristics. Thus, the aim of this work is to analyze chromosomally, for the first time, six species belonging to three araneid genera from Brazil. The specimens of *Alpaida leucogramma* (White 1841), *Alpaida truncata* (Keyserling 1865), *Alpaida veniliae* (Keyserling 1865), *Parawixia kochi* (Taczanowski 1873), *Parawixia velutina* (Taczanowski 1878) and *Wagneriana* sp. were collected in Parque Nacional de Ilha Grande and in the municipality of Rio Claro. The gonads were treated with colchicine and hypotonic solution before fixation with Carnoy I solution. The results were $2n♂ = 24$ ($11II+X_1X_2$) in *A. leucogramma* and *P. velutina*, and $2n♂ = 22$ ($10II+X_1X_2$) in *A. truncata*, *A. veniliae*, *P. kochi* and *Wagneriana* sp. When the chromosomal morphologies were established, we observed telocentric chromosomes in all specimens save one female specimen of *P. velutina*. The univalent sex chromosomes were easily recognized on diplotenes. The unpaired metacentric element found in one female specimen of *P. velutina* with $2n = 25$ probably arises by centric fusion/fission. Araneidae is a megadiverse family composed of ~3000 species distributed mainly in the tropics; thus the analysis of more species may provide new insights about orb-weaver chromosome evolution.

Keywords: Sex chromosome system, meiosis, mitosis, chromosome, spider

Despite the fact that the spider family Araneidae Clerck 1757 is the third largest, comprising 3006 species in 168 genera (Platnick 2011), only 65 species from 20 genera have ever been cytogenetically characterized (approximately 2% of the total diversity). Although relatively few araneid species have been studied, they represent the third most studied family from the cytogenetical point of view. However, there are many gaps remaining in our knowledge of this diverse group. Of the 65 species studied, at least 50 show a diploid complement of $2n♂ = 24$, with 22 autosomal elements and an X_1X_2 sex chromosome system. The remaining species' diploid number varies from $2n♂ = 13$ to $2n♂ = 49$. Moreover, while the X_1X_2 sex chromosome system is the most common within the family, the type X occurred with a smaller frequency, and the $X_1X_2X_3$ and XY types were each observed in only one species. The chromosomal morphology for most species is classified as acro/telocentric (summarized in Table 1).

The araneid genera *Alpaida* O. Pickard-Cambridge 1889, *Parawixia* F.O. Pickard-Cambridge 1904, and *Wagneriana* F.O. Pickard-Cambridge 1904, represent genera that have never been cytogenetically evaluated. The genus *Alpaida* is limited to Central and South America and comprises 140 species (Platnick 2011). *Alpaida leucogramma* (White 1841) and *Alpaida veniliae* (Keyserling 1865) are distributed from Panama to Argentina (Platnick 2011). *Alpaida truncata* (Keyserling 1865) is found from Mexico to Argentina (Levi 1988; Platnick 2011). The genus *Parawixia* includes 31 species, which occur almost exclusively in Central and South America. *Parawixia kochi* (Taczanowski 1873) is found from Trinidad to Brazil and *Parawixia velutina* (Taczanowski 1878) is recorded from Colombia to Argentina (Levi 1992; Platnick 2011). The genus *Wagneriana* comprises 41 species distributed mainly in Central and South America (Levi 1991; Platnick 2011).

Cytogenetical studies of spiders are scarce, thus hypotheses regarding chromosomal evolution are difficult to formulate or test. The aim of this study is to characterize for the first time the species *A. leucogramma*, *A. truncata*, *A. veniliae*, *P. kochi*, *P. velutina*, and *Wagneriana* sp. in relation to the diploid number, chromosomal morphology, and type of sex chromosome system. Our study represents the first cytogenetical characterization of Neotropical araneid species.

METHODS

Spiders were collected in the Parque Nacional de Ilha Grande, Paraná, Brazil, and in the municipality of Rio Claro, São Paulo, Brazil (Table 2), between 2008 and 2009. After the gonads were dissected, the specimens were deposited in the arachnological collection of the Instituto Butantan (IBSP, curator I. Knysak) in the state of São Paulo, Brazil.

Chromosomal preparations and standard staining were performed according to Araujo et al. (2008). The mitotic and meiotic cells were photographed using a digital-imaging capture system coupled to a light microscope, and the chromosomal morphology was classified according to Levan *et al.* (1964).

RESULTS

Alpaida.—The analyses of spermatogonial metaphases and spermatocytes I of *A. leucogramma* showed $2n♂ = 24 = 22+X_1X_2$ telocentric chromosomes that decrease gradually in length (Fig. 1A) and 11 autosomal bivalents plus two sex

Table 1.—List of species of the family Araneidae studied cytogenetically. Valid names extracted from Platnick (2010); cited as = species name used in the original paper; $2n$ =male diploid number (without parentheses), or female diploid number (between parentheses); SCS = sex chromosome system. T = telocentric; A = acrocentric; H = holocentric; M = metacentric; Sm = submetacentric; St = subtelocentric.

Araneidae Simon 1895						
Valid name	Cited as	$2n$	SCS	Chromosome morphology	Collection site	Bibliography
Acusilas coccineus Simon 1895	----	24	X_1X_2	----	Japan	Matsumoto 1977
Alenatea fuscocolorata (Bösenberg & Strand 1906)	*Araneus fuscocoloratus*	24	X_1X_2	$22A+X_1X_2A$	Japan	Suzuki 1954
Alpaida leucogramma (White 1841)	----	24	X_1X_2	$22T+X_1X_2T$	Brazil	Present work
Alpaida truncata (Keyserling 1865)	----	22	X_1X_2	----	Brazil	Present work
Alpaida veniliae (Keyserling 1865)	----	22	X_1X_2	----	Brazil	Present work
Araneus angulatus Clerck 1757	*Aranea angulata*	24	X_1X_2	$22A+X_1X_2A$	Finland	Hackman 1948
Araneus diadematus Clerck 1757	*Aranea diademata*	24	X_1X_2	$22A+X_1X_2A$	Finland	Hackman 1948
A. diadematus Clerck 1757	*Aranea diademata*	24	X_1X_2	----	----	Sokolov 1960
A. diadematus Clerck 1757	*Araneus diadema*	24	X_1X_2	----	India	Mittal 1960
A. diadematus Clerck 1757	*Araneus diadema*	24	X_1X_2	$22A+X_1X_2A$	India	Mittal 1966
Araneus ejusmodi Bösenberg & Strand 1906	*Lithyphantes dubius*	24	X_1X_2	$22A+X_1X_2A$	Japan	Suzuki, 1954
Araneus lathyrinus (Holmberg 1875)	*Metepeira lathyrina*	24	X_1X_2	$22A+X_1X_2A$	----	Diaz & Saez 1966
Araneus mitificus (Simon 1886)	*Araneus nutificus*	24	----	----	----	Suzuki 1951a
A. mitificus (Simon 1886)	----	24	X_1X_2	----	Japan	Suzuki 1951b
Araneus quadratus Clerck 1757	*Aranea reaumuri*	24	X_1X_2	$22T+X_1X_2T$	Germany	Pätau 1948
Araneus ventricosus (L. Koch 1878)	----	48	X_1X_2	----	----	Suzuki 1951a
A. ventricosus (L. Koch 1878)	----	46	X_1X_2	$44A+X_1X_2A$	Japan	Suzuki 1951b
A. ventricosus (L. Koch 1878)	----	32	----	----	----	Zhang & Tong 1990
A. ventricosus (L. Koch 1878)	*Aranea ventricosus*	49	$X_1X_2X_3$	----	----	Youju et al. 1993
Araneus sp.	----	24	X_1X_2	----	----	Suzuki 1950
Araneus sp.	----	24	X_1X_2	$22A+X_1X_2A$	Japan	Suzuki 1951b
Araneus sp.	----	24	X_1X_2	----	India	Mittal 1960
Araniella curcubitina (Clerck 1757)	*Aranea curcubitina*	24	X_1X_2	$22A+X_1X_2A$	Finland	Hackman 1948
Argiope amoena L. Koch 1878	----	24	X_1X_2	----	----	Suzuki 1950
A. amoena L. Koch 1878	----	24	X_1X_2	----	Japan	Suzuki 1951b
Argiope bruennichi (Scopoli 1772)	*Argiope bruennichii*	26	----	----	----	Zhang & Tong 1990
Argiope catenulata (Doleschall 1859)	----	24	XY	H	Philippines	Amalin 1988
A. catenulata (Doleschall 1859)	----	24	XY	H	Philippines	Amalin et al. 1992
Argiope luzona (Walckenaer 1841)	----	24	----	M+Sm+St	Philippines	Carandang & Barrion 1994a
Argiope minuta Karsch 1879	*Argiope shillongensis*	24	X_1X_2	----	India	Datta & Chatterjee 1983
A. minuta Karsch 1879	*Argiope shillongensis*	24	X_1X_2	$22T+X_1X_2T$	India	Datta & Chatterjee 1988
A. minuta Karsch 1879	*Argiope shillongensis*	24	----	----	----	Carandang & Barrion 1994b
Argiope pulchella Thorell 1881	----	24	X_1X_2	$22A+X_1X_2A$	India	Bole-Gowda 1958
Cyclosa atrata Bösenberg & Strand 1906	----	24	X_1X_2	----	----	Suzuki 1951a
C. atrata Bösenberg & Strand 1906	----	24	X_1X_2	----	Japan	Suzuki 1951b
Cyclosa bifida (Doleschall 1859)	----	24	X_1X_2	----	India	Datta & Chatterjee 1983
C. bifida (Doleschall 1859)	----	24	X_1X_2	$22A+X_1X_2A$	India	Datta & Chatterjee 1984
C. bifida (Doleschall 1859)	----	24	X_1X_2	$22T+X_1X_2T$	India	Datta & Chatterjee 1988
Cyclosa confraga (Thorell 1892)	----	24	X_1X_2	----	India	Mittal 1960

Table 1.—Continued.

Araneidae Simon 1895						
Valid name	Cited as	2*n*	SCS	Chromosome morphology	Collection site	Bibliography
C. confraga (Thorell 1892)	----	24	X_1X_2	$22A+X_1X_2A$	India	Mittal 1966
Cyclosa conica (Pallas 1772)	----	24	X_1X_2	----	India	Mittal 1960
C. conica (Pallas 1772)	----	24	X_1X_2	$22A+X_1X_2A$	India	Mittal 1966
Cyclosa octotuberculata Karsch 1879	*Cyclosa 8-tuberculata*	24	X_1X_2	----	----	Suzuki 1949
C. octotuberculata Karsch 1879	*Cyclosa 8-tuberculata*	24	X_1X_2	----	----	Suzuki 1950
C. octotuberculata Karsch 1879	----	24	X_1X_2	$22A+X_1X_2A$	Japan	Suzuki 1951b
Cyclosa sedeculata Karsch 1879	----	24	X_1X_2	$22A+X_1X_2A$	Japan	Suzuki 1954
Cyclosa spirifera Simon 1889	----	24	X_1X_2	----	India	Datta & Chatterjee 1983
C. spirifera Simon 1889	----	24	X_1X_2	$22A+X_1X_2A$	India	Datta & Chatterjee 1984
C. spirifera Simon 1889	----	24	X_1X_2	$22T+X_1X_2T$	India	Datta & Chatterjee 1988
Cyclosa walckenaeri (O. Pickard-Cambridge 1889)	*Cyclosa walckenaerii*	24	X_1X_2	----	India	Mittal 1960
C. walckenaeri (O. Pickard-Cambridge 1889)	*Cyclosa walckenaerii*	24	X_1X_2	$22A+X_1X_2A$	India	Mittal 1966
Cyclosa sp.	----	24	X_1X_2	----	India	Mittal 1960
Cyrtophora cicatrosa (Stoliczka 1869)	----	24	X_1X_2	----	India	Parida & Sharma 1987
C. cicatrosa (Stoliczka 1869)	----	24	X_1X_2	----	India	Sharma & Parida 1987
Cyrtophora citricola (Forsskål 1775)	----	24	X_1X_2	----	India	Mittal 1960
C. citricola (Forsskål 1775)	----	24	X_1X_2	$22A+X_1X_2A$	India	Mittal 1966
C. citricola (Forsskål 1775)	----	(26)	$X_1X_1X_2X_2$	$22T+X_1X_1X_2X_2T$	India	Datta & Chatterjee 1988
Cyrtophora feai (Thorell 1887)	*Araneus feae*	24	X_1X_2	$22A+X_1X_2A$	India	Bole-Gowda 1958
Eriovixia poonaensis (Tikader & Bal 1981)	*Neoscona poonaensis*	24	X_1X_2	----	India	Datta & Chatterjee 1983
E. poonaensis (Tikader & Bal 1981)	*Neoscona poonaensis*	24	X_1X_2	----	India	Sharma & Parida 1987
E. poonaensis (Tikader & Bal 1981)	*Neoscona poonaensis*	24	X_1X_2	$22T+X_1X_2T$	India	Datta & Chatterjee 1988
Eustala emertoni (Banks 1904)	----	24	----	----	USA	Tugmon et al. 1990
Eustala sagana (Keyserling 1893)	*Araneus saganus*	(26)	----	26A	Japan	Suzuki 1954
Eustala sp.	*Eustela* sp.	24	X_1X_2	----	India	Mittal 1961
Gasteracantha cancriformis (Linnaeus 1758)	----	31	X	----	Philippines	Amalin 1988
Gasteracantha hasselti C.L. Koch 1837	*Gasteracantha hasseltii*	16	X_1X_2	----	India	Datta & Chatterjee 1983
G. hasselti C.L. Koch 1837	*Gasteracantha hasseltii*	16	X_1X_2	$14T+X_1X_2T$	India	Datta & Chatterjee 1988
Gasteracantha kuhli C.L. Koch 1837	*Gasteracantha leucomelaena*	16	X_1X_2	----	India	Datta & Chatterjee 1983
G. kuhli C.L. Koch 1837	*Gasteracantha reucomelaena*	16	X_1X_2	$14T+X_1X_2T$	India	Datta & Chatterjee 1988
Larinia directa (Hentz 1847)	----	24	X_1X_2	----	India	Mittal 1960
L. directa (Hentz 1847)	----	24	X_1X_2	$22A+X_1X_2A$	India	Mittal 1966
Larinia sp.	----	24	X_1X_2	----	India	Mittal 1960
Larinia sp.	----	24	X_1X_2	----	India	Sharma et al. 1960
Larinia sp.	----	24	X_1X_2	$22A+X_1X_2A$	India	Mittal 1966
Lariniaria argiopiformis (Bösenberg & Strand 1906)	*Larinia punctifera*	24	X_1X_2	$22A+X_1X_2A$	Japan	Suzuki 1954
Larinioides cornutus (Clerck 1757)	*Aranea foliata*	24	X_1X_2	$22A+X_1X_2A$	Finland	Hackman 1948
L. cornutus (Clerck 1757)	*Araneus cornutus*	24	----	----	----	Qingtao et al. 1998

Table 1.—Continued.

Valid name	Cited as	2*n*	SCS	Chromosome morphology	Collection site	Bibliography
Araneidae Simon 1895						
L. cornutus (Clerck 1757)	*Araneus cornutus*	23	----	----	----	Qingtao et al. 1998
L. cornutus (Clerck 1757)	*Larinioides cornuta*	24	X_1X_2	----	----	Qingtao et al. 1999
L. cornutus (Clerck 1757)	*Larinioides cornuta*	23	X_1X_2	----	----	Qingtao et al. 1999
Larinioides patagiatus (Clerck 1757)	*Aranea dumetorum*	14	X_1X_2	$10M+2A+X_1X_2A$	Finland	Hackman 1948
Larinioides sclopetarius (Clerck 1757)	*Epeira sclopetaria*	23	X	----	----	Berry 1906
L. sclopetarius (Clerck 1757)	*Epeira sericata*	24	X_1X_2	----	----	Painter 1914
Neoscona achine (Simon 1906)	----	24	X_1X_2	----	India	Datta & Chatterjee 1983
N. achine (Simon 1906)	----	24	X_1X_2	$22T+X_1X_2T$	India	Datta & Chatterjee 1988
Neoscona adianta (Walckenaer 1802)	*Araneus japonicus*	24	----	----	----	Suzuki 1951a
N. adianta (Walckenaer 1802)	*Araneus japonicus*	24	X_1X_2	----	Japan	Suzuk 1951b
Neoscona arabesca (Walckenaer 1841)	----	24	X_1X_2	----	India	Mittal 1960
N. arabesca (Walckenaer 1841)	----	24	X_1X_2	$22A+X_1X_2A$	India	Mittal 1966
N. arabesca (Walckenaer 1841)	*Neoscona minina*	14	----	10M+2Sm+2T	----	Amalin et al. 1993
N. arabesca (Walckenaer 1841)	----	21	X	----	USA	Doan & Paliulis 2009
Neoscona mukerjei Tikader 1980	----	24	X_1X_2	----	India	Sharma & Parida 1987
Neoscona oaxacensis (Keyserling 1864)	----	14	----	10M+4Sm	----	Amalin et al. 1993
Neoscona pavida (Simon 1906)	*Araneus pavidus*	24	X_1X_2	----	India	Mittal 1960
Neoscona punctigera (Doleschall 1857)	*Araneus opima*	24	X_1X_2	----	----	Suzuki 1951a
N. punctigera (Dolschall 1857)	*Araneus opima*	24	X_1X_2	----	Japan	Suzuki 1951b
Neoscona scylla (Karsch 1879)	*Araneus scylla*	24	X_1X_2	----	----	Suzuki 1951a
N. scylla (Karsch 1879)	*Araneus scylla*	14	X_1X_2	$10M+2A+X_1X_2A$	Japan	Suzuki 1951b
Neoscona theisi (Walckenaer 1841)	----	23	X	----	Philippines	Amalin 1988
Neoscona sp.	----	14	X_1X_2	----	India	Parida & Sharma 1987
Neoscona sp.	----	24	X_1X_2	----	India	Parida & Sharma 1987
Neoscona sp.	----	13	X	----	India	Parida & Sharma 1987
Neoscona sp.	----	13	X	----	India	Sharma & Parida 1987
Neoscona sp.	----	13	X	----	India	Sharma & Parida 1987
Neoscona sp.	----	24	X_1X_2	----	India	Sharma & Parida 1987
Nuctenea umbratica (Clerck 1757)	*Aranea sexpunctata*	24	X_1X_2	$22A+X_1X_2A$	Finland	Hackman 1948
N. umbratica (Clerck 1757)	*Araneus umbratica*	24	X_1X_2	----	India	Mittal 1960
N. umbratica (Clerck 1757)	*Araneus umbratica*	24	X_1X_2	$22A+X_1X_2A$	India	Mittal 1966
Parawixia kochi (Taczanowski 1873)	----	22	X_1X_2	----	Brazil	Present work
Parawixia velutina (Taczanowski 1878)	----	24	X_1X_2	$22T+X_1X_2T$	Brazil	Present work
Singa hamata (Clerck 1757)	----	24	X_1X_2	$22A+X_1X_2A$	Russia	Gorlov et al. 1995
Stroemiellus stroemi (Thorell 1870)	*Zilla stroemi*	24	X_1X_2	$22A+X_1X_2A$	Finland	Hackman 1948
Wagneriana sp.	----	22	X_1X_2	----	Brazil	Present work

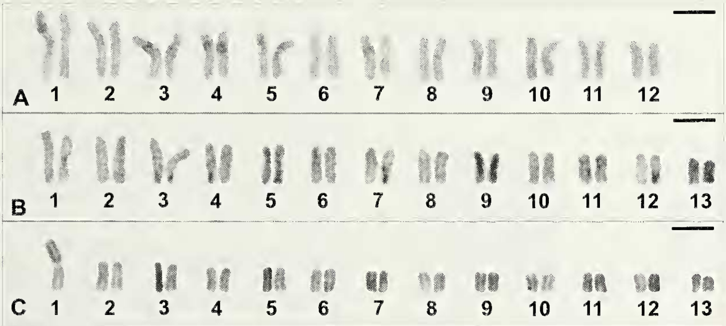
Table 2.—List of species cytogenetically analyzed in the present study, with the number of specimens (males and females) studied, number of cells analyzed (Cells), collecting location and deposit number of the specimens in the arachnological collection of the Instituto Butantan (IBSP). The number of specimen of *P. velutina* with $2n$ = 25 is in bold letters.

Species	♂	♀	Cells	Collection site	IBSP
Alpaida leucogramma	1	–	30	Campus UNESP (22°23′33.97″S, 47°32′37.39″W), Rio Claro, São Paulo, Brazil	53028
Alpaida truncata	2	–	107	São Francisco Island (24°00′32.40″S, 54°09′52.18″W), Paraná, Brazil	153928, 151186
Alpaida veniliae	1	1	27	Xambrê lake margins (23°52′48.64″S, 54°0′12.25″W), Altônia, Paraná, Brazil	145318, 145319
Parawixia kochi	1	–	62	São Francisco Island (24°00′32.40″S, 54°09′52.18″W), Paraná, Brazil	123695
Parawixia velutina	3	3	348 (224 – $2n$=24 and 26; 124 – $2n$=25)	São Francisco Island (24°00′32.40″S, 54°09′52.18″W), Paraná, Brazil	123717, 154838, 154847, 154844, 154842, **151176**
Wagneriana sp.	1	–	287	São Francisco Island (24°00′32.40″S, 54°09′52.18″W), Paraná, Brazil	152927

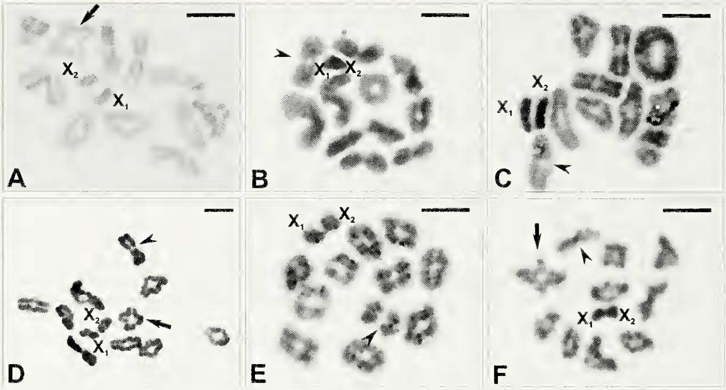
univalents ($11II+X_1X_2$) (Fig. 2A). The sex chromosomes X_1 and X_2 are the smallest of the complement (Figs. 1A, 2A). Diplotenes of *A. truncata* (Fig. 2B) and *A. veniliae* (Fig. 2C) showed meiotic formulae comprising 10 autosomal bivalents and two sexual univalents ($10II+X_1X_2$) for both species, indicating a diploid number of $2n♂ = 22$, composed of 20 autosomes and the X_1X_2 sex chromosomes. In *A. truncata* the X_1 and X_2 sex univalents always appear to be side-by-side (Fig. 2B). In all *Alpaida* studied, the sex chromosomes are positive heteropycnotic in a number of diplotene nuclei (Fig. 2A–C). The majority of autosomal bivalents showed only one terminal chiasm, but bivalents with an interstitial chiasm also occurred (Fig. 2A–C). A female pachytene cell of *A. veniliae* revealed 12 chromosomal bivalents (Fig. 3A), indicating the occurrence of $2n♀ = 24$, comprising 20 autosomes and the $X_1X_1X_2X_2$ sex chromosome system. The chromosomal morphology was not established in *A. truncata* and *A. veniliae*, due to the lack of mitotic or metaphase II cells.

Parawixia.—The observation of spermatogonial diplotenes of *P. kochi* and *P. velutina* showed 10 and 11 autosomal bivalents, respectively (Fig. 2D, E). Both species have two sexual univalents that always appear side by side and correspond to the X_1 and X_2 chromosomes (Fig. 2D, E). Therefore, the meiotic formula of *P. kochi* is $10II+X_1X_2$, corresponding to a diploid number of $2n♂ = 22$. In *P. velutina* the meiotic formula is $11II+ X_1X_2$, indicating a diploid complement of $2n♂ = 24$. This complement was confirmed by the presence of $2n♀ = 26$ in oogonial metaphases of two females of *P. velutina* (Fig. 1B). Mitotic metaphases of one female specimen of *P. velutina* showed an astonishing characteristic; the diploid number is $2n♀ = 25$, with a large unpaired metacentric chromosome (Fig. 1C), contrasting with the telocentric elements of the karyotype. In both analyzed *Parawixia* species, the autosomal bivalents showed only one interstitial or terminal chiasm. Differential pycnosis was not observed in any chromosome of the complement in *Parawixia* species (Fig. 2D, E). Only one metaphase II nucleus was found in *P. kochi*, showing $n = 10$ autosomes and no sex chromosomes (Fig. 3B).

Wagneriana.—The analyses of male diplotene cells in *Wagneriana* sp. revealed 10 autosomal bivalents and two



Figures 1A–C.—Karyotypes of araneid species. A. *Alpaida leucogramma*, with $2n♂ = 24 = 22+X_1X_2$ telocentric elements; B. *Parawixia velutina*, $2n♀ = 26 = 22+X_1X_1X_2X_2$ telocentric chromosomes; C. *Parawixia velutina*, heterozygote specimen, $2n♀ = 25 = 21+X_1X_1X_2X_2$, with 24 telocentrics and one unpaired metacentric chromosome, probably result of a centric fusion. Scale = 10 μm.

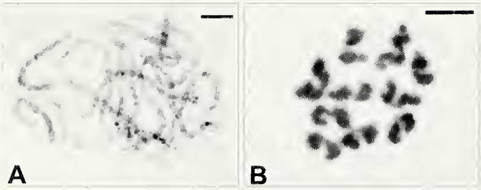


Figures 2A–F.—Male diplotenes of araneid species. A. *Alpaida leucogramma*, 11 autosomal bivalents plus two sex chromosomes ($11II+X_1X_2$); B, C. *Alpaida truncata* and *Alpaida veniliae*, respectively, $10II+X_1X_2$. D. *Parawixia kochi*, $10II+X_1X_2$. E. *Parawixia velutina*, $11II+X_1X_2$. F. *Wagneriana* sp., $10II+X_1X_2$. Arrows indicate interstitial chiasma and arrowheads indicate terminal chiasma. Scale = 10µm.

sexual univalents ($10II+X_1X_2$) (Fig. 2F), suggesting a diploid number of 2n♂ = 22. The autosomal bivalents presented only one terminal or interstitial chiasma. The sex chromosomes were positive heteropycnotic in relation to the autosomes and always appeared closely associated (Fig. 2F). It was not possible to identify the chromosomal morphology due to the lack of mitotic metaphases or metaphase II.

DISCUSSION

The acquisition and analysis of cytogenetic data may show more difficulties than the acquisition of morphological or molecular data; i.e., the specimens must be kept alive until dissection and must present cells in division (Araujo et al. 2005). However, molecular sequencing, despite becoming more accessible, is not available for many researchers out of the major research centers. Thus, chromosomal data can be used 1) as a complement to molecular/morphological data, 2) when molecular data are not available, or 3) when morphological characteristics are not effective for identifying species (i.e., cryptic species). Moreover, the chromosome number has already been used in taxonomical descriptions of spider species (see Maddison 1996). The differences in male diploid number between *A. truncata*, *A. veniliae* (2*n* = 22), and *A. leucogramma* (2*n* = 24), as well as between *P. kochi* (2*n* = 22) and *P. velutina* (2*n* = 24) could be useful as a cytotaxonomical character, which, together with genital characteristics, can be used to identify these closely related, sympatric species.



Figures 3A–B.—Early and late meiotic stages in araneid spiders. A. *Alpaida veniliae* female pachytene with 12 bivalents, indicating 2*n*♀ = 24. B. *Parawixia kochi* male metaphase II, *n* = 10 chromosomes. Scale = 10 µm.

The 2*n*♂ = 22 diploid number has now been observed for the first time in Araneidae (see Table 1) for the species *A. truncata*, *A. veniliae*, *P. kochi* and *Wagneriana* sp. It is interesting to note that Levi (1992), in a taxonomic review, indicated that the genera *Parawixia*, *Alpaida*, and *Wagneriana* shared (together with *Acanthepeira* Marx 1883, *Wixia* O. Pickard-Cambridge 1882, *Eriophora* Simon 1864, *Verrucosa* McCook 1888 and others) some external morphological putative synapomorphies (see Levi 1992 for details). The presence of species with 2*n* = 22 exclusively in these genera among araneids could be considered further evidence of their close relationship. Unfortunately, there is no cytogenetical data on other closely related genera such as *Acanthepeira*, *Wixia*, *Eriophora*, *Verrucosa* and *Edricus* O. Pickard-Cambridge 1890.

Of the three araneid genera studied here, only *Alpaida* was included in the phylogenetic hypothesis of Scharff & Coddington (1997), whose results do not corroborate the hypothesis of Levi (1992), since the genera *Acanthepeira*, *Eriophora* and *Verrucosa* appeared far from *Alpaida*, that arises within clade 63, which also includes *Bertrana* Keyserling 1884, *Enacrosoma* Mello-Leitão 1932, *Eustala* Simon 1895, *Wixia* and *Acacesia* Simon 1895 (Scharff & Coddington 1997). Of these genera, the only one that has a description of cytogenetical data is *Eustala*, which possesses the most common chromosome number in araneids, 2*n*♂ = 24 (see Table 1). Thus, a more extensive cytogenetical study of Araneidae representatives is necessary in order to understand the chromosomal evolution process that resulted in the smaller chromosome number (2*n* = 22) in *Parawixia* and *Alpaida*.

Araneids, in general, have exclusively acro/telocentric chromosomes (Table 1). Exceptions are 1) two *Argiope*

Audouin 1826 species with $2n♂ = 24$ biarmed or holocentric chromosomes (Amalin 1988; Amalin et al. 1992; Carandang & Barrion 1994a) and 2) one *Larinioides* Caporiacco 1934 and three *Neoscona* Simon 1864 species that have karyotypes composed of $2n♂ = 14$ chromosomes, with 10 metacentric elements (Hackman 1948; Suzuki 1951b; Amalin et al. 1993). According to Suzuki (1951b), the second case probably was a karyotype with $2n♂ = 24$ acro/telocentric chromosomes that suffered centric fusions to form a karyotype with $2n♂ = 14$, with 10 metacentrics originated by the fusions of 20 acro/telocentric elements and 4 non-fusioned acro/telocentrics.

In the *Parawixia velutina* specimen with $2n♀ = 25$, the metacentric element probably arose through a Robertsonian fusion (centric fusion), where the centromeric regions of two acro/telocentric chromosomes fuse to form a single meta/submetacentric chromosome, generating a heterozygosis condition. Frequently, chromosomal changes produce reproductive barriers when they cause problems during meiosis in heterozygotes, leading to reduced fertility, owing to the formation of a trivalent during meiosis and unbalanced segregation. However, during meiosis segregation both unbalanced and balanced gametes could be formed (Sumner 2003).

In the case of the heterozygote specimen of *P. velutina* $2n♀ = 25$, the meiosis can proceed in two ways: 1) generate unbalanced gametes that are eliminated during gametogenesis, do not fertilize or produce unviable embryos; or 2) the fusioned metacentric pairs with their two homologous telocentrics to form a trivalent and properly generate balanced gametes, some containing the metacentric and others containing the two telocentrics. Unfortunately, because the heterozygote individual was a female, it was not possible to observe meiotic pairing.

Centric fusion is a very common evolutionary change and has been reported in most groups of organisms (White 1973). This type of rearrangement, as presently verified in one specimen of *P. velutina* with $2n♀ = 25$, was observed in the heterozygous condition in one male specimen of the salticid *Evarcha hoyi* (Peckham & Peckham 1883) that presents $2n♂ = 25$ (with one unpaired metacentric autosome), contrasting with the $2n♂ = 26$ (without metacentric autosome) verified in other specimens in the same study (Maddison 1982). In many cases, however, the homozygous condition rapidly reached fixation, with the original telo/acrocentric elements disappearing from the population (White 1973), as seems to be the case for the previously cited *Larinioides* and *Neoscona* species with $2n = 14$ described by Hackman (1948), Suzuki (1951b) and Amalin et al. (1993).

On the other hand, *Gasteracantha hasselti* C.L. Koch 1837 and *Gasteracantha kuhli* C.L. Koch 1837 possess $2n = 16$ exclusively acro/telocentric chromosomes (Datta & Chatterjee, 1983, 1988). According to Datta & Chatterjee (1988), tandem/centric fusion followed by pericentric inversion from a karyotype with $2n♂ = 24$ acro/telocentric chromosomes is involved in the origin of the karyotype with $2n = 16$; and, owing to the pericentric inversions, there are no metacentric chromosomes in the karyotype with $2n = 16$, despite the chromosome number reduction.

In this study we found four species with $2n♂ = 22$, suggesting that this diploid number could be more common in araneids than previously thought. This diploid number is also very frequent in the Theridiidae Sundeval 1833b, another araneoid family (Araujo et al. 2010). We also show that even within a genus (i.e., *Parawixia* and *Alpaida*), some species are $2n♂ = 24$, while others are $2n♂ = 22$, suggesting that the rearrangements involved in the conversion from 24 to 22 chromosomes or vice versa are relatively common among araneoid spiders.

Thus, this study presents cytogenetical data of Neotropical orb-weavers for the first time. The results were slightly in disagreement with those recorded for Old World species, and further studies are required to evaluate whether the observed pattern persists for other Neotropical species. The cytogenetical characterization of representatives of the genera closely related to those analyzed in the present study (*Parawixia*, *Alpaida* and *Wagneriana*), especially Araneines sensu Scharff & Coddington (1997), and *Acanthepeira*, *Wixia*, *Eriophora*, *Verrucosa* and *Edricus*, as suggested by Levi (1992), is strongly recommended as they seem to be essential for understanding chromosome evolution in araneids.

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